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Tittel:

Optical imaging of colorectal cancer

Optisk avbilding av kolorektal kreft

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Signatur:
Marianne W. Wulff

Navn i blokkbokstaver:
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Optical imaging of colorectal cancer

Field of the invention

The present invention provides contrast agents that for optical imaging of colorectal cancer (CRC) in patients. The contrast agents may be used in diagnosis of CRC, for follow up of progress in disease development, and for follow up of treatment of CRC.

The present invention also provides new methods of optical imaging of CRC in patients, for diagnosis and for follow up of disease development and treatment of CRC.

Description of related art

Colorectal cancer (CRC) is one of the most frequent malignant diseases in the western civilization. More than 100 000 new cases of CRC are diagnosed every year in US and the disease will kill more than 56 000 Americans in 2003. This makes CRC the fourth most commonly diagnosed cancer disease and it is the second leading cause of cancer death in US after lung cancer.

The peak incidence of CRC generally occurs after the age of 60 years. CRC is more common in the western world than in underdeveloped countries. There might be several reasons for this including; general life expectancy, genetic susceptibility and diet. It is suggested that intake of fat and red meat have a negative effect on the incidence of CRC while intake of fiber might decrease the risk for CRC.

Most of the CRC incidents are adenocarcinomas. The sizes of the lesions are normally in the range from a few millimetres to several centimetres, and the lesions are not evenly distributed through the lower part of the gastrointestinal system. Most lesions are found in the rectum. CRC cells remain normally superficial for a long time and will slowly invade the deeper layers in the intestinal wall and later through the intestinal wall. A majority of the patients with advanced colorectal cancer develop liver metastasis during the course of the disease.

Several therapeutic drugs are today used for treatment of CRC. These include Eloxatine® (oxaliplatin), Camptosar® (irinotecan), OncoVAX, Tomudex® (raltitrexed), TS-1, Futulon (doxifluridine) and Xeloda (capecitabine). Several therapeutic products are in late development including Thalomide® (thalidomide), Avastin® (bevacizumab), NeuTrexin® (trimetrexate), Panorex® (edrecolomab) and Erbitux (cetuximab).

The prognosis for the patient is very dependent on the progress of the disease. With no metastasis and localization of the tumor(s) to bowel mucosa the 5-year survival prognosis is

80%, while patients with advanced CRC with distant metastasis have a low (<5%) 5-year survival prognosis.

With the prognosis it is critical to diagnose CRC at an early stage before the disease invades deeper layers of the intestinal wall and before the patients develop liver metastasis. The clinical symptoms of CRC are often non-specific. However, typical symptoms can be discoloured stool (blood in stool); abdominal pain, weight loss, fever and diarrhoea. The methods used to diagnose CRC include coloscopy, fecal occult blood testing, sigmoideoscopy and double-contrast barium colonography. CT colonography is comparable to colonoscopy for detection of colorectal polyps equal to or larger than 10 mm. The American Cancer Society and others have suggested performing CRC screening of the population or parts of the population. Several clinical studies conclude that screening for CRC is cost effective compared to no screening. Although screening methods for early detection of CRC is available, many patients have CRC diagnosed at a late stage and have poor prognosis. There are several advantages related to the methods used to screen and diagnose CRC today. However, colonoscopy has always a risk of perforation, faecal occult blood testing results in very many false positive results based on other sources of blood like for example haemorrhoids. No methods, including x-ray methods, are CRC specific and therefore result in many false positive results (e.g. polyps). Existing diagnostic methods for diagnosis of CRC not only result in many false positive results, but a problem is that the use of these methods also results in many false negative results. None of these methods are useful for safe early diagnosis of CRC at the stage where the disease is superficial. The most specific method might be positron emission tomography (PET) with fluorodeoxyglucose (FDG), but this method is expensive and should be reserved for equivocal cases.

A study of recently published literature on CRC shows that there is a medical need for a cheap, simple and safe method for diagnosis of CRC at an early stage.

US 6,455,688 claims a method for diagnosing CRC by determining the expression of a gene encoding a specific sequence (CJA8).

US 6,326,148 provides a method of screening for colon carcinoma cells in a sample by determining the presence of increased copy number of chromosome 20q.

US 6,316,272 suggests a method of diagnosis of CRC related to a specific nucleic acid sequence.

US 6,187,591 claims a screening test for colorectal cancer whereby a marker is detected in rectal mucus. The marker is detected in the mucus deposited on a support using Schiff's reagent.

US 6,150,100 claims a method for diagnosis of tumors of the gastrointestinal tract such as colorectal tumors based on determination of the genomic instability at 5 selected microsatelite locl.

US 6,149,581 claims a device and method for access to the colon and small bowel of a patient.

US 5,416,025 claims a method for detecting CRC by adding an enzyme to a mucus sample to detect a specific disaccharide marker.

US 5,380,647 claims a test for detecting carcinoembryonic antigen (CEA) in stool. CEA is indicator of the presence of CRC.

US 4,996,298 claims a new method for diagnosis of CRC based on glycoprotein as a marker for CRC.

US 4,857,457 claims a method for detecting the presence of precancer or cancer of the large intestine by assaying the presence of a disaccharide in a mucus sample.

JP II-225800 claims a method for detecting colon cancer using fluorescent material. The method relates to telomerase, however, the method is an in vitro method and does not suggest contrast agents.

Although there are several patents and scientific publications on optical imaging using contrast agents with fluorescent properties in the infrared or near infrared part of the spectrum, there are no documents focusing on diagnosis of CRC. As pointed out CRC is still a challenge to diagnose and treat.

There is still need for improved diagnostic methods, especially for contrast agents for optical diagnosis of CRC in an early stage with good reliability. We have surprisingly discovered that the use of the combination of optical imaging methods and new contrast agents fulfill these requirements.

Summary of the invention

In view of the needs of the art the present invention provides a contrast agent for optical imaging with affinity for an abnormally expressed biological target associated with CRC.

The invention is also described in the claims.

The following definitions will be used throughout the document:

Contrast agent: Molecular moiety used for enhancement of image contrast *in vivo* comprising at least one element that interacts with light in the ultraviolet, visible or near infrared part of the electromagnetic spectrum.

Optical imaging: Any method that forms an image for diagnosis of disease, follow up of disease development or for follow up of disease treatment based on interaction with light in the electromagnetic spectrum from ultraviolet to near infrared radiation. Optical imaging includes all methods from direct visualization without use of any device and use of devices such as various scopes, catheters and optical imaging equipment, for example computer based hardware for tomographic presentations.

Diagnosis: In the context of this invention, diagnosis includes screening of selected populations, early detection, biopsy guidance, characterisation, staging, grading, therapy efficacy monitoring, long-term follow-up of relapse and surgical guidance.

CRC tissue: Any tissue in the colon or rectum that shows changes associated with neoplasia or preneoplasia, and including metastates from colorectal cancer at other sites in the body.

Overexpressed target: A receptor, an enzyme or another molecule or chemical entity that is present in a higher amount in diseased tissue than in normal tissue.

Downregulated target: A receptor, an enzyme or another molecule or chemical entity that is present in a lower amount in diseased tissue than in normal tissue.

Mutated target: A protein in CRC tissue that is altered as a result of a germline or sotatic mutation, and including alterations resulting from differential splicing of RNA and changes in post-translational modifications, particularly glycosylation patterns, but not limited to these types of alterations.

Detailed description of the invention

A first aspect of the present invention is contrast agents for optical imaging of CRC.

The contrast agent has affinity for an abnormally expressed target associated with endometriosis. By abnormally expressed, is meant that the target is either downregulated, mutated or overexpressed.

CRC tissue containing a downregulated target may be identified by a low amount of bound imaging agent compared to normal tissue. In this situation, the amount of imaging agent should be less than 50 % of that in normal tissue, preferably less than 10 %.

Targets that are mutated in CRC tissue may be identified by lack of binding of an imaging agent that does bind to normal tissue; alternatively, the imaging agent might be directed specifically towards the mutated target, and binding to normal tissue would be minimal. Mutations in CRC-associated genes are often non-random. For instance, more than 90 % of mutations in the K-ras gene observed in CRC occur at codon 12 or 13. Somatic mutations in the important adenomatous polyposis coli (APC) gene commonly occur at codons 1309-1311 or codon 1450.

Preferred contrast agents according to the invention, has affinity for an overexpressed target associated with CRC. Preferred targets are those targets that are more than 50 % more abundant in CRC tissue than in surrounding tissue. More preferred targets are those targets that are more than two times more abundant in CRC tissue than in surrounding tissue. The most preferred targets are those targets that are more than 5 times more abundant in CRC tissue than in surrounding tissue.

Relevant groups of targets are receptors, enzymes, nucleic acids, proteins, lipids, other macromolecules like for example lipoproteins and glycoproteins. The targets may be located in the vascular system, in the extracellular space, associated with cell membranes or located intracellularly.

The following biological targets are among the preferred targets for contrast agents for optical imaging of CRC:

Adhesion molecules and adhesion-associated molecules:
Beta-catenin, E-cadherin (CDH1 gene), adenomatous polyposis coli protein (APC), p120-catenin, CD44-standard, CD44-6v, CD44-9v, 67-kDa laminin receptor.

Antigens:
Human leukocyte antigen-B18 and human leukocyte antigen-DQ5, tissue polypeptide antigen (TPA) or tissue polypeptide-specific antigen (TPS), Small intestinal mucin antigen (SIMA), CA15.3, CA 19-9, CA 72-4, CYFRA 21-1, CAM 17.1, CEA, TPS, CA 72-4, MUC-1, tumour-associated antigen L6, HLA-A, CA-195, CA-242, beta HCG, AFP, CA125.

Enzymes:
alpha-Methylacyl-CoA racemase, aminopeptidase N/CD13, carcinogen metabolising enzymes, arachidonic acid metabolism, enzymes responsible for polyamine metabolism,

CDC25B phosphatase, COX-1, cyclooxygenase-2 (COX-2), Cytochrome P450 2A6 (CYP2A6), Glutathione S-transferase, gamma-glutamylcysteine synthetase (gamma-GCS) and DT-diaphorase, Guanylyl cyclase C, matrix metalloproteinases and their inhibitors (especially, MMP-2, MMP-7, MMP-9, stromelysin-3), mitochondrial aspartate-aminotransferase, phosphoglucomutase, plasminogen-related molecules, thymidylate synthase, Tumour-associated trypsin inhibitor (TATI), u-PA, prostaglandin E synthase and Cathepsins, typically Cathepsin B and human aspartyl (asparginyl) Beta-hydroxylase (HAAH).

Signal molecules and their receptors:
Beta-HCG, c-erbB, and VEGF, c-Myc, gastrin, CCK(B)-R, Gastrin, Bradeion (septin family gene), WNT7A, WNT7B, insulin-like growth factor 2, benzodiazepine receptor, Her-2, VEGF receptors, EGF receptors.

Tumour suppressor proteins, oncogenes, apoptosis-related proteins:
Adenomatous polyposis coli protein (APC), Bax, Bcl-2, beta-catenin/T cell factor-4 (Tcf-4), Groucho proteins, proteins in K-ras cascade, nm23, p53, K-ras, Deleted in Colorectal Cancer (DCC), c-erbB2, Survivin.

Others:
L-plastin, the human homologue of yeast ribosomal protein S28, the B-cell translocation gene, AXIN2, Chromogranin A, synaptophysin, syntaxin1, VAMP2, SNAP25, alpha/beta-SNAP, Clusterin (apolipoprotein j), ITF-2, PPARdelta, Cystatin-like metastasis-associated protein, EBP50, etheno (epsilon)-DNA adducts (e.g., via trans-4-hydroxy-2-nonenal), keratin 5, Ki-67, Mib-1, proliferating cell nuclear antigen, osteopontin, p27 (kip-1), proliferating cell nuclear antigen (PCNA), WAF1, p34cdc2, cyclins B1 and D1, SBA2, sigma B3 protein, transcription factor nuclear factor-kappa beta (NF-kappaB) and Hypoxia-inducible factor.

Among the most preferred targets for contrast agents for optical imaging of CRC are:
COX-2, beta-catenin, E-cadherin, various kinases, benzodazepine receptor, Her-2, MMPs, cyclins, P53, thymidylate synthase, VEGF receptors, EGF receptors, K-ras, adenomatous polyposis coli protein and Cathepsin B.

Generally, any targets that have been identified as possible targets for agents for treatment of CRC might be potential targets also in optical imaging.

The contrast agents, according to the present invention, are comprised of a targeting moiety that binds to an abnormally expressed target in CRC tissue, and an optical reporter.

The preferred contrast agents of the present invention are molecules with relatively low molecular weights. The molecular weight of preferred contrast agents is below 10000 Daltons, more preferably below 7000 Daltons.

Thus viewed from one aspect the present invention provides a contrast agent of formula I:

V-L-R (I)

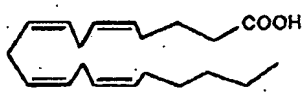
wherein V is one or more vector moieties having affinity for one or more abnormally expressed target in CRC tissue, L is a linker moiety or a bond and R is one or more moieties detectable in optical imaging.

Hence, the contrast agents of the present invention comprise at least one optical reporter molecule linked to a molecule sub-unit (vector) that binds to an abnormally expressed target in CRC tissue.

The agents of formula I have three characteristic components: a vector (V); a linker (L); and a reporter (R). The vector must have the ability to target the contrast agent to a region of CRC, the reporter must be detectable in an optical imaging procedure and the linker must couple vector to reporter, at least until the reporter has been delivered to the region of CRC and preferably until the imaging procedure has been completed.

The vector can for example be selected from the following group of compounds: peptides, peptidomimetics, oligonucleotides, oligosaccharides, fat-related compounds and traditional organic molecules. The targeting part of the contrast agent should preferably have a molecular weight of less than 4500 Daltons and more preferably less than 2500 Daltons. Below are some examples of vectors having affinity for CRC related abnormally expressed targets:

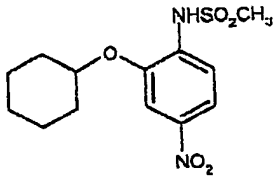
Vectors for COX-2: Arachidonic acid



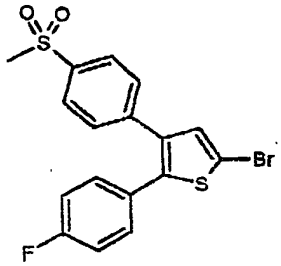
Arachidonic acid is the endogenous substrate for COX-2.

Other vectors for COX-2 are exogenous compounds that bind to COX-2 for example so-called COX-2 inhibitors. The chemical classes of the main COX-2 inhibitors are shown in WO 02/07721.

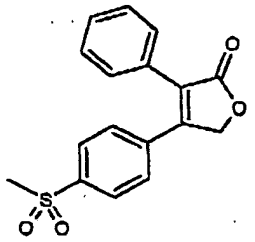
Such ligands include:



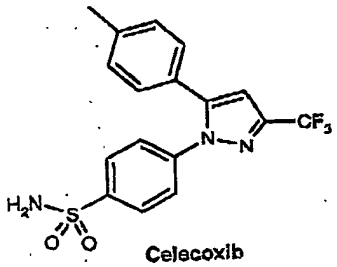
NS-398



DuP-697



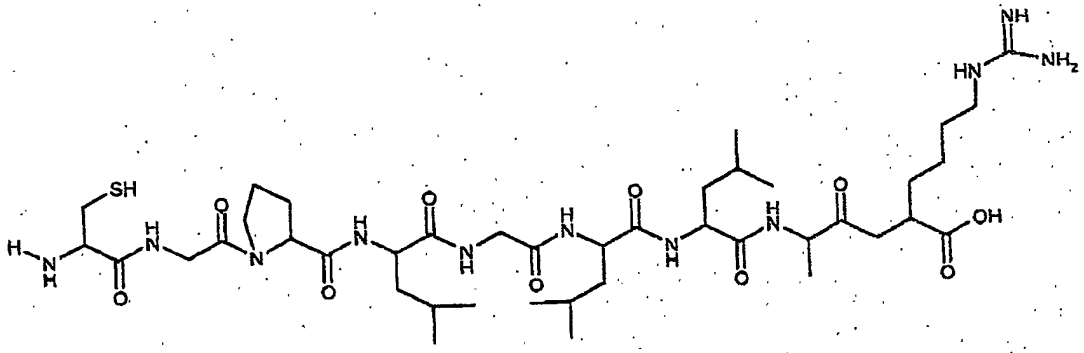
Rofecoxib



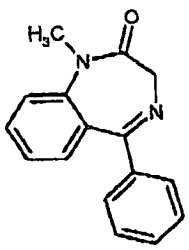
Celecoxib

Vectors for MMP-7:

Peptide sequence: Cys-Gly-Pro-Leu-Gly-Leu-Leu-Ala-Arg-OH



Vector for benzodiazepine receptor:



A wide variety of linkers can be used. The linker component of the contrast agent is at its simplest a bond between the vector and the reporter moieties. In this aspect the reporter part of the molecule is directly bound to the molecule sub-unit that binds to the abnormally expressed target. More generally however the linker will provide a mono- or multi-molecular skeleton covalently or non-covalently linking one or more vectors to one or more reporters, e.g. a linear, cyclic, branched or reticulate molecular skeleton, or a molecular aggregate, with in-built or pendant groups which bind covalently or non-covalently, e.g. coordinatively, with the vector and reporter moieties. The linker group can be relatively large in order to build into the contrast agent optimal size or optimal shape or simply to improve the binding characteristics for the contrast agent to the abnormally expressed target in CRC tissue.

Thus linking of a reporter unit to a desired vector may be achieved by covalent or non-covalent means, usually involving interaction with one or more functional groups located on the reporter and/or vector. Examples of chemically reactive functional groups which may be employed for this purpose include amino, hydroxyl, sulfhydroxyl, carboxyl and carbonyl groups, as well as carbohydrate groups, vicinal diols, thioethers, 2-aminoalcohols, 2-aminothiols, guanidinyl, imidazolyl and phenolic groups.

Covalent coupling of reporter and vector may therefore be effected using linking agents containing reactive moieties capable of reaction with such functional groups. Examples of reactive moieties capable of reaction with sulfhydryl groups include a-haloacetyl compounds of the type X-CH_2CO- (where X=Br, Cl or I) which show particular reactivity for sulfhydryl groups but which can also be used to modify imidazolyl, thioether, phenol and amino groups. N- Maleimide derivatives are also considered selective towards sulfhydryl groups, but may additionally be useful in coupling to amino groups under certain conditions. Reagents such as 2-iminothiolane which introduce a thiol group through conversion of an amino group, may be considered as sulfhydryl reagents if linking occurs through the formation of disulphide bridges. Thus reagents which introduce reactive disulphide bonds into either the reporter or the vector may be useful, since linking may be brought about by disulphide exchange between the vector and reporter; examples of such reagents include Ellman's reagent (DTNB), 4,41-dithiodipyridine, methyl- 3-nitro-2-pyridyl disulphide and methyl-2-pyridyl disulphide.

Examples of reactive moieties capable of reaction with amino groups include alkylating and acylating agents. Representative alkylating agents include:
i)α-haloacetyl compounds, which show specificity towards amino groups in the absence of reactive thiol groups and are of the type X-CH_2CO- (where X=Cl, Br or I);
ii) N-maleimide derivatives, which may react with amino groups either through a Michael type reaction or through acylation by addition to the ring carbonyl group;
iii) aryl halides such as reactive nitrohaloaromatic compounds;
iv) alkyl halides;
v) aldehydes and ketones capable of Schiff's base formation with amino groups. the adducts formed usually being stabilised through reduction to give a stable amine;
vi) epoxide derivatives such as epichlorohydrin and bisoxiranes,which may react with amino, sulfhydryl or phenolic hydroxyl groups;
vii) chlorine-containing derivatives of s-triazines, which are very reactive towards nucleophiles such as amino, sufhydryl and hydroxy groups;
viii) aziridines based on s-triazine compounds detailed above, which react with nucleophiles such as amino groups by ring opening;
ix) squaric acid diethyl esters
X) α-haloalkyl ethers, which are more reactive alkylating agents than normal alkyl halides because of the activation caused by the ether oxygen atom.

Representative amino-reactive acylating agents include:
i) isocyanates and isothiocyanates, particularly aromatic derivatives, which form stable urea and thiourea derivatives respectively and have been used for protein crosslinking;
ii) sulfonyl chlorides, which may be useful for the introduction of a fluorescent reporter group into the linker; iii) Acid halides;
iv) Active esters such as nitrophenylesters or N- hydroxysuccinimidyl esters;
v) acid anhydrides such as mixed, symmetrical or N- carboxyanhydrides;
vi) other useful reagents for amide bond formation;
vii) acylazides, e.g. wherein the azide group is generated from a preformed hydrazide derivative using sodium nitrite; viii) azlactones attached to polymers such as bis- acrylamide;
ix) Imidoesters , which form stable amidines on reaction with amino groups.

Carbonyl groups such as aldehyde functions may be reacted with weak protein bases at a pH such that nucleophilic protein side-chain functions are protonated. Weak bases include 1,2-aminothiols such as those found in N-terminal cysteine residues, which selectively form stable 5-membered thiazolidine rings with aldehyde groups. Other weak bases such as phenyl hydrazones may be used. Aldehydes and ketones may also be reacted with amines to form Schiff's bases, which may advantageously be stabilised through reductive amination.

Alkoxylamino moieties readily react with ketones and aldehydes to produce stable alkoxamines.

Examples of reactive moieties capable of reaction with carboxyl groups include diazo compounds such as diazoacetate esters and diazoacetamides, which react with high specificity to generate ester groups. Carboxylic acid modifying reagents such as carbodiimides, which react through O-acylurea formation followed by amide bond formation, may also usefully be employed; linking may be facilitated through addition of an amine or may result in direct vector-receptor coupling. Useful water soluble carbodiimides include 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimid'e (CMQ and i-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Other useful carboxylic acid modifying reagents include isoxazolium derivatives such as Woodwards reagent K; chloroformates such as p-nitrophenylchloroformate; carbonyldiimidazoles such as 1,1-carbonyldiimidazole; and N-carbalkoxydihydroquinolines such as N- (ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline. Other potentially useful reactive moieties include vicinal diones such as p-phenylenediglyoxal, which may be used to react with guanidinyl groups; and diazonium salts, which may undergo electrophilic substitution reactions. Compounds are readily prepared by treatment of aryl diamines with sodium nitrite in acidic solutions.

It will be appreciated that functional groups in the reporter and/or vector may if desired be converted to other functional groups prior to reaction, e.g. to confer additional reactivity or selectivity. Examples of methods useful for this purpose include conversion of amines to carboxylic acids using reagents such as dicarboxylic anhydrides; conversion of amines to thiols using reagents such as N-acetylhomocysteine thiolactone, S- acety1mercaptosuccinic anhydride, 2-iminothiolane or thiol-containing succinimidyl derivatives; conversion of thiols to carboxylic acids using reagents such as a-haloacetates; conversion of thiols to amines using reagents such as ethylenimine or 2-bromoethylamine; conversion of carboxylic acids to amines using reagents such as carbodiimides followed by diamines; and conversion of alcohols to thiols using reagents such as tosyl chloride followed by transesterification with thioacetate and hydrolysis to the thiol with sodium acetate.

The reporter moieties in the contrast agents of the invention may be any moiety capable of detection either directly or indirectly in an optical imaging procedure. The reporter might be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter. More preferably the reporter is a dye such as a chromophore or a fluorescent compound. The dye part of the contrast agent can be any dye that interacts with light in the electromagnetic spectrum with wavelenghts from the ultraviolet light to the near infrared. Preferably the contrast agent of the invention has fluorescent properties.

Preferred organic chromophoric and fluorophoric reporters include groups having an extensive delocalized electron system, eg. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyriliup dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, napthoquinones, indathrenes, phthaloylacridones, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, bis(dithiolene) complexes, bis(benzene-dithiolate) complexes, iodoaniline dyes, bis(S,O-dithiolene) complexes. Fluorescent proteins, such as green fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium, samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots).

Particular examples of chromophores which may be used include fluorescein, sulforhodamine 101 (Texas Red), rhodamine B, rhodamine 6G, rhodamine 19, indocyanine green, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, Marina Blue, Pacific Blue, Oregon Green 488, Oregon Green 514, tetramethylrhodamine, and Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and Alexa Fluor 750.

Particularly preferred are dyes which have absorption maxima in the visible or near infrared region, between 400 nm and 3 μm, particularly between 600 and 1300 nm.

Several relevant targets for CRC are enzymes. A contrast agent for optical imaging of CRC for targeting an enzyme can be an enzyme contrast agent substrate that can be transformed to a contrast agent product possessing different pharmacokinetic and/or pharmacodynamic properties from the contrast agent substrate. In this embodiment the invention provides contrast agent substrates having affinity for an abnormally expressed enzyme, wherein the contrast agent substrate changes pharmacodynamic and/or pharmacokinetic properties upon a chemical modification into a contrast agent product in a specific enzymatic transformation, and thereby enabling detection of areas of disease upon a deviation in the enzyme activity from the normal. Typical differences in pharmacodynamic and/or pharmacokinetic properties can be binding properties to specific tissue, membrane penetration properties, protein binding and solubility issues.

Alternatively, if the abnormally expressed target for diagnosis of CRC is an enzyme, the contrast agent for optical imaging can be a dye molecule that directly binds to the enzyme. The contrast agent will have affinity for the abnormally expressed enzyme, and this may be used to identify tissue or cells with increased enzymatic activity.

In a further aspect of the invention the contrast agent changes dye characteristics as a result of an enzymatic transformation. For example, a fluorescent dye reporter of the contrast agent is quenched (no fluorescence) by associated quencher groups, until an enzymatic cleavage takes place, separating the dye from the quencher groups and resulting in fluorescence at the site of the abnormally expressed enzyme.

Another aspect of this part of the invention is that the dye may change colour, as e.g. a change in absorption and/or emission spectrum, as a result of an enzymatic transformation.

If the abnormally expressed target for diagnosis of CRC is a receptor or another non-catalytical target, the contrast agent for optical imaging can bind directly to the target and normally not change the dye characteristics.

Another aspect of the invention is contrast agents for optical imaging of CRC characterized by having affinity for more than one abnormally expressed target related to the disease. Such contrast agents can have two or more different vectors or molecular subunits that target two or more different abnormally expressed targets.

Another possibility according to the present invention is that the contrast agent has one vector that is able to bind to more than one overxpressed target in CRC.

A contrast agent according to the present invention might also have more than one vector of same chemical composition that bind to the abnormally expressed biological target.

Another aspect of the present invention is contrast agents for optical imaging of CRC characterized in that the contrast agent comprises more than one dye molecular sub-unit. These dye sub-units might be similar or different from a chemical point of view. Preferred contrast agents have less than 6 dye molecular sub-units.

The preferred contrast agents of the present invention are soluble in water. This means that the preferred contrast agents have a solubility in water at pH 7.4 of at least 1 mg/ml.

The contrast agents of the present invention can be identified by random screening, for example by testing of affinity for abnormally expressed targets of a library of dye labeled compounds either prepared and tested as single compounds or by preparation and testing of mixture of compounds (a combinatorial approach).

The contrast agents of the present invention can also be identified by use of technology within the field of intelligent drug design. One way to perform this is to use computer-based techniques (molecular modelling or other forms of computer-aided drug design) or use of

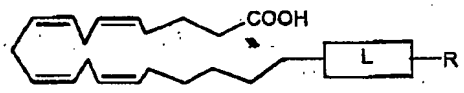
knowledge about natural and exogenous ligands (vectors) for the abnormally expressed targets. The sources for exogenous ligands can for example be the chemical structures of therapeutic molecules for targeting the same target. One typical approach here will be to bind the dye chemical sub-unit to the targeting vector so that the binding properties of the vector are not reduced. This can be performed by linking the dye at the far end away from the pharmacophore centre (the active targeting part of the molecule).

The contrast agents of the invention are preferably not endogenous substances alone. Some endogenous substances, for instance estrogen have certain fluorescent properties in themselves, but they are not likely to be sufficient for use in optical imaging. Endogenous substances combined with an optical reporter however, falls within the contrast agents of the invention.

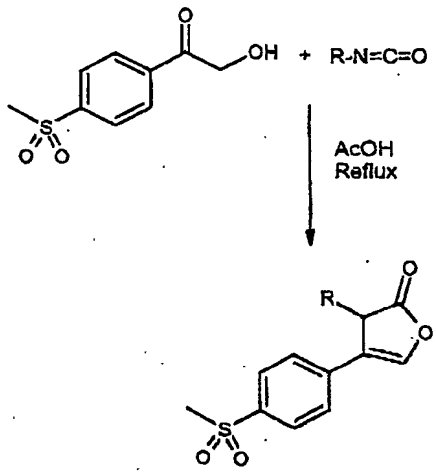
The contrast agents will be useful with optical imaging modalities and measurement techniques including, but not limited to: luminescence imaging; endoscopy; fluorescence endoscopy; optical coherence tomography; transmittance imaging; time resolved transmittance imaging; confocal imaging; nonlinear microscopy; photoacoustic imaging; acousto-optical imaging; spectroscopy; reflectance spectroscopy; interferometry; coherence interferometry; diffuse optical tomography and fluorescence mediated diffuse optical tomography (continuous wave, time domain and frequency domain systems), and measurement of light scattering, absorption, polarisation, luminescence, fluorescence lifetime, quantum yield, and quenching.

Some examples on contrast agent molecules for optical imaging of CRC according to the invention are shown below:

Contrast agents with affinity for mapping of COX-2:

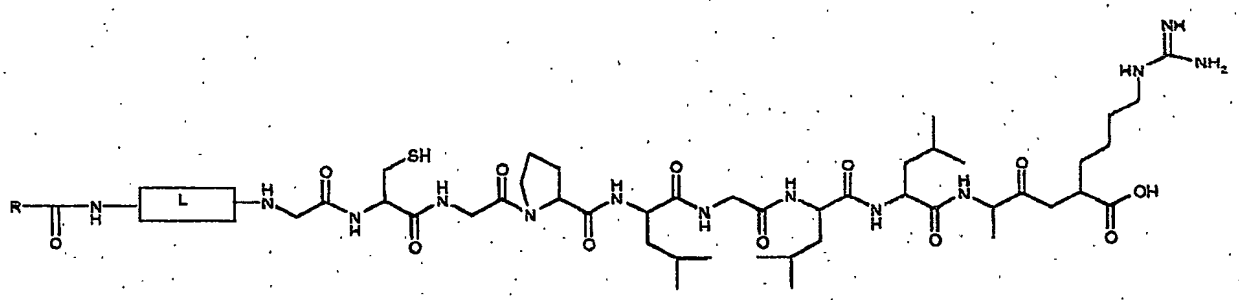


and

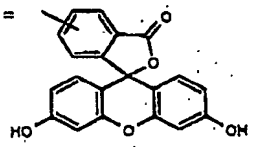


Wherein R is any reporter according to the present invention; for example fluorescein, and L is a linker.

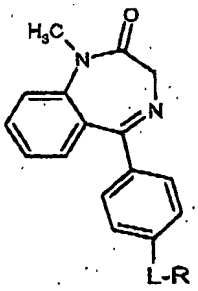
Contrast agent for mapping of matrix metalloproteinase wherein the vector peptide is linked to fluorescein through a linker:



R =



Contrast agent with affinity for binding to benzodiazepine receptor:



Wherein L is a linker and R is one of the mentioned reporters.

A further embodiment is use of contrast agents of the invention for optical imaging of CRC, that is, for diagnosis of CRC, for use in follow up the progress in CRC development or for follow up the treatment of CRC.

Still another embodiment of the invention is a method of optical imaging for diagnosis of CRC using the contrast agents as described.

Still another embodiment of the invention is a method of optical imaging to follow up the progress of CRC development and to follow up the treatment of CRC.

One aspect of these methods is to administer the present contrast agents and follow the accumulation and elimination directly visually during surgery. Another aspect of these methods is to administer the present contrast agents and perform visual diagnosis through a colonoscope.

Still another aspect of the present invention is to administer the present contrast agents and perform the image diagnosis using computerized equipment as for example a tomograph.

Still another embodiment of the invention is use of a contrast agent as described for the manufacture of a diagnostic agent for use in a method of optical imaging of CRC involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said body.

Still another embodiment of the invention is pharmaceutical compositions comprising one or more contrast agents as described or pharmaceutically acceptable salts thereof for optical imaging for diagnosis of CRC, for follow up progress of CRC development or for follow up the treatment of CRC. The diagnostic agents of the present invention may be formulated in conventional pharmaceutical or veterinary parenteral administration forms, e.g. suspensions, dispersions, etc., for example in an aqueous vehicle such as water for injections. Such compositions may further contain pharmaceutically acceptable diluents and excipients and formulation aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc. The most preferred formulation is a sterile solution for intravascular administration or for direct injection into area of interest. Where the agent is formulated in a ready-to-use form for parenteral administration, the carrier medium is preferably isotonic or somewhat hypertonic.

The dosage of the optical diagnostic agents of the invention will depend upon the clinical indication, choice of contrast agent and method of administration. In general, however dosages will be between 10μg and 5 grams for an adult human.

While the present invention is particularly suitable for methods involving parenteral administration of the contrast agent, e.g. into the vasculature or directly into an organ of muscle tissue, intravenous administration being especially preferred, it is also applicable where administration is not via a parenteral route, e.g. where administration is transdermal, nasal, sub-lingual or is into an externally voiding body cavity, e.g. the gi tract, the bladder, the uterus or the vagina. The present invention is deemed to extend to cover such administration.

The following examples are illustrative only and not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

Examples:

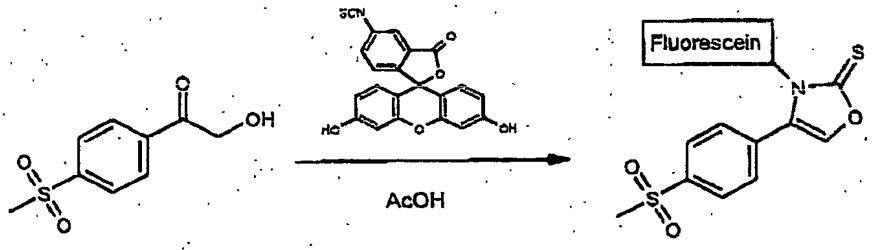
Example 1 Contrast agent for mapping of COX-2 activity. Synthesis of COX-2 ligand coupled to fluorescein.

Step 1

2-Hydroxy-1-(4-methanesulfonylphenyl)ethanone is prepared from 2-bromo-1-(4-methanosulfonylphenyl)ethanone according to C. Puig et al in J.Med.Chem 2000,43, 214-223.

Step 2

A solution of 2-hydroxy-1-(4-methanosulfonylphenyl) ethanone (1.50 g, 7 mmol) and fluorescein isocyanate isomer I (2.72 g, 7 mmol) is heated in DMF at 120°C for 5 hours. The mixture is cooled, DMF evaporated off and acetic acid (25ml) is added. The mixture is refluxed for 10 hours. The acetic acid is evaporated and the resulting mixture is purified on silica using chloroform/methanol as eluent.



Example 2 Contrast agent for mapping of matrix metalloproteinase (MMP). Synthesis of fluorescein–Cys-Gly-Pro-Leu-Gly-Lev-Leu-Ala-Arg-OH linker conjugate

Step 1

The peptide component was synthesised on an ABI 433A automatic peptide synthesiser starting with Fmoc–Arg(Pmc)–wang resin on a 0.1 mmol scale using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. An aliquot of the peptide resin was then transferred to a clean round bottom flask an N-methyl morpholine (1 mmol) in DMF (5 ml) added followed by chloroacetyl chloride (1 mmol). The mixture was gently shaken until Kaiser test negative. The resin was extensively washed with DMF.

Step 2

5(6)–carboxyfluorescein (188 mg, 0.5 mmol) and dicyclohexylcarbodiimide (113 mg, 0.55 mmol) are dissolved in DMF (20 ml). The mixture is stirred for 2 hours and cooled to 0°C. A solution of hexamethylenediamide (116 mg, 1 mmol) and DMAP (30 mg) in DMF is added and the mixture is stirred at ambient temperature for 72 hours. The solution is evaporated and the conjugate between carboxyfluorescein and hexamethylene-amine is isolated as monoamide by chromatography (silica, chloroform and methanol).

Step 3

The resin from step 1 is suspended in DMF (5 ml) and amide-amine conjugate from step 2 (0.5 mmol) pre-dissolved in DMF (5ml) containing triethylamine (0.5 mmol) is added. The mixture is heated to 50°C for 16 hours then excess reagents filtered off, following extensive washing with DMF, DCM and diethyl ether then air drying. The product is treated with TFA containing TIS (5%), H_2O (5%), and phenol (2.5%) for 2 hours.

Excess TFA is removed *in vacuo* and the peptide is precipitated by the addition of diethyl ether. The crude peptide conjugate is purified by preparative HPLC C C-18, acetonitril, TFA, water).

Example 3 Contrast agent for binding to benzodiazepine receptor

Step 1

Nitrazepam is reduced to the corresponding 7-aminonitrazepam using standard conditions zink in aqueous hydrochloric acid, catalytic hydrogenation or other reduction agents.

Step 2

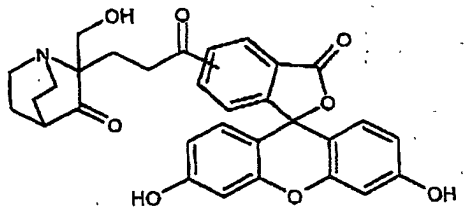
5(6) Carboxyfluorescein (1 mmol) and dicyclohexylcarbodiimide (1 mmol) are dissolved in DMF (30 ml). The mixture is stirred for 2 hours at ambient temperature. A solution of 7-aminnitrazepam (1 mmol) and DMAP (20 mg) in DMF (10 ml) is added and the mixture is

evaporated and the conjugate between 7-aminonitrazepam and 5(6) carboxyfluorescein is isolated by chromatography (silica, chloroform/methanol).

Example 4 Contrast agent for binding to p53 oncoprotein

Step 1 Synthesis of 2,2-bis(hydroxymethyl)-1-aza-bicyclo[2,2,2,]octan-3-one 3-quinuclidinone hydrochloride (Aldrich Q 190-5) (1 mmol) is dissolved in methanol-water (1:1, 30 ml). An aqueous solution of formaldehyde (37 %, 2.5 mmol) and sodium hydroxide (1.5 mmol) are added. The mixture is stirred for 12 hours at 50°C. The solvents are evaporated and the title compound isolated as free base using flash chromatography (silica, ethylacetate/chloroform, hexane).

Step 2.



5(6)-carboxyfluorescein (0.1 mmol) and dicyclohexyl carbodiimide (0.11 mmol) are dissolved in DMF. The mixture is stirred for 3 hours and cooled to 0 °C. A solution of 2,2-bis(hydrozymethyl)-1-azabicyclo[2,2,2] octane-3-one (0.5 mmol) and DMAP (10 mg) in DMF is added and the mixture is stirred at ambient temperature for 72 hours. The solution is evaporated and the contrast agent is isolate by flash chromatography (silica, ethyl acetate/hexane).

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Claims:

1. A contrast agent for optical imaging with affinity for an abnormally expressed biological target associated with CRC.

2. A contrast agent as claimed in claim 1 with molecular weight below 10000 Daltons.

3. A contrast agent as claimed in claim 1 or 2 of formula I

V-L-R, (I)

wherein V is one or more vector moieties having affinity for an abnormally expressed target in CRC, L is a linker moiety or a bond and R is one ore more moieties detectable in optical imaging.

4. A contrast agent as claimed in any of claims 1 to 3 comprising a contrast agent substrate, wherein the target is an abnormally expressed enzyme, such that the contrast agent changes pharmacodynamic properties and/or pharmacokinetic properties upon a chemical modification from a contrast agent substrate to a contrast agent product upon a specific enzymatic transformation.

5. A contrast agent as claimed in any of claims 1 to 4 having affinity for any of the receptors selected from COX-2, beta-catenin, E-cadherin, various kinases, benzodazepine receptor, Her-2, MMPs, cyclins, P53, thymidylate synthase, VEGF receptors, EGF receptors, K-ras, adenomatous polyposis coli protein and Cathepsin B.

6. A contrast agent as claimed in claims 3 or 4 wherein V is selected from peptides, peptoid moieties, oligonucleotides, oligosaccharides and fat-related compounds.

7. A contrast agent as claimed in any of claims 3-6 wherein R is a dye that interacts with light in the wavelength region from the ultraviolet to the infrared part of the electromagnetic spectrum.

8. A pharmaceutical composition for optical imaging for diagnosis of CRC, for follow up of progress of CRC development or for follow up of treatment of CRC, comprising a contrast agent as defined in any of claims 1 to 7 together with at least one pharmaceutically acceptable carrier or excipient.

9. Use of a contrast agent as claimed in any of claims 1 to 7 for the manufacture of a diagnostic agent for use in a method of optical imaging of CRC involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said subject.

10. A method of generating an optical image of an animate subject involving administering a contrast agent to said subject and generating an optical image of at least a part of said subject to which said contrast agent has distributed, characterized in that as said contrast agent is used a contrast agent as defined in an any of claims 1 to 7.

11. Method as claimed in claim 10 for diagnosis of CRC, for follow up of the progress of CRC development or follow up of treatment of CRC using a contrast agent as defined in any of claims 1 to 7.

12. Use of a contrast agent as defined in any of claim 1 to 7 for optical imaging of CRC.

Abstract

The invention provides contrast agents for optical imaging of CRC in patients. The contrast agents may be used in diagnosis of CRC, for follow up of progress in disease development, and for follow up of treatment of CRC. Further, the invention provides methods for optical imaging of CRC in patients.

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